

PB87-100715

Effects of Synthetic Polyelectrolytes on
Selected Aquatic Organisms

(U.S.) Environmental Protection Agency
Duluth, MN

1986



U.S. Department of Commerce
National Technical Information Service

NTIS

TECHNICAL REPORT DATA		
(Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA/600/J-86/128	2.	3. PB87-100715
4. TITLE AND SUBTITLE Effects of Synthetic Polyelectrolytes on Selected Aquatic Organisms. (Journal Version)	5. REPORT DATE 1986	
	6. PERFORMING ORGANIZATION CODE	
7. AUTHOR(S) Kenneth E. Biesinger and G.N. Stokes	8. PERFORMING ORGANIZATION REPORT NO.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. EPA, Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, MN 55804	10. PROGRAM ELEMENT NO.	
	11. CONTRACT/GRANT NO.	
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory U.S. Environmental Protection Agency Office of Research and Development Duluth, MN 55804	13. TYPE OF REPORT AND PERIOD COVERED	
	14. SPONSORING AGENCY CODE EPA-600/3	
15. SUPPLEMENTARY NOTES J. Water Pollut. Control Fed. 58(3):207-213 (1986). (March).		
16. ABSTRACT The acute toxicity of several polyelectrolytes to daphnids (<u>Daphnia magna</u>), fathead minnows (<u>Pimephales promelas</u>), gammarids (<u>Gammarus pseudolimnaeus</u>) and midges (<u>Paratanytarsus parthenogeneticus</u>) was tested. Most nonionic and anionic polyelectrolytes were not toxic at 100 mg/l which was the highest concentration tested. Two of 15 cationic polyelectrolytes were not toxic at 100 mg/l, whereas the toxicity of the others ranged from 0.09 to 70.71 mg/l. A cationic polyelectrolyte was tested in microcosms using five aquatic animals and 10 algae; various adverse effects were observed. An anionic polyelectrolyte and three different kinds of clay detoxified a cationic polyelectrolyte for daphnids and fathead minnows. Exposure of fathead minnows to a cationic polyelectrolyte resulted in severe gill damage.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT Release to public.	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 8
	20. SECURITY CLASS (This page) Unclassified	22. PRICE



Effects of synthetic polyelectrolytes on selected aquatic organisms

Kenneth E. Biesinger, Gertrude N. Stokes

Synthetic polyelectrolytes (polymers) are used extensively to promote flocculation in potable water and municipal and industrial wastewaters. The pulp and paper, textile, fiberglass, glass and ceramic, paint, metal and metal finishing, mining and petroleum industries use polyelectrolytes for product recovery and waste treatment. Despite their extensive use and discharge into most water in developed countries, few papers have been published on toxicity or other adverse effects of polyelectrolytes to aquatic organisms. Biesinger *et al.*¹ exposed selected aquatic animals to six polyelectrolytes and reported acute toxicities of 0.29 mg/L to 345 mg/L. Safe concentrations of polyelectrolytes for fathead minnows (*Pimephales promelas*) were reported from 2 to 1000 mg/L.² Brocksen³ stated that 1 mg/L or less of a cationic polymer was acutely toxic to rainbow trout (*Salmo gairdneri*). The Kobunshi Gyoshuzai Konwakai (KGK)⁴ reported acute toxicities to rainbow trout (0.42 mg/L to 7180 mg/L) for 12 cationic polymers.

The acute toxicity of several polyelectrolytes to daphnids (*Daphnia magna*), fathead minnows (*Pimephales promelas*), gammarids (*Gammarus pseudolimnaeus*), and midges (*Paratanytarsus parthenogeneticus*) was tested in this study. A microcosm test using five aquatic animals and 10 algal species was also conducted. In addition, tests were made to determine the detoxification of cationic polyelectrolytes by anionic polyelectrolytes and by red, montmorillonite, and kaolinite clays. Gill damage as a result of exposure to a cationic polyelectrolyte was evaluated by microscopic examination of fathead minnow gill tissue.

METHODS AND MATERIALS

Daphnids, midges, fathead minnows, ostracods (*Cyprinotus* sp.), rotifers (*Philodina* sp.), protozoans (*Hypotricha* sp.), amphipods (*Hyalella azteca*), and algae (*Anabaena cylindrica*, *Ankistrodesmus* sp., *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Lyngbya* sp., *Nitzschia kuetzingiana*, *Scenedesmus obliquus*, *Selenastrum capricornutum*, *Stigeoclonium* sp. and *Ulothrix* sp.) were obtained from cultures maintained at the U. S. Environmental Protection Agency (EPA) Environmental Research Laboratory at Duluth, Minn. Gammarids were collected from the Eau Claire River near Gordon, Wisc.

Acute bioassays were conducted with fathead minnows and daphnids at a polyelectrolyte concentration of 100 mg/L. If the chemical was toxic at this concentration to either or both of these animals, it was then tested using less sensitive gammarids. Some of these compounds were tested using midges. To obtain LC₅₀s, five to eight concentrations of polyelectrolytes, along with controls, were tested. Test organisms, stage of development,

containers, water volume, number of animals, number tested at each concentration, and test duration are given in Table 1. For tests with gammarids, a stainless steel screen was placed at the bottom of each beaker to help prevent cannibalism. All tests were conducted at 20° ± 1°C. Daphnid tests were made in reconstituted water (Standard Methods 801D⁵) at a hardness of 161.5 ± 4.3 mg/L, alkalinity of 121.5 ± 5.6 mg/L, conductivity of 520 ± 39 µmhos/cm, and pH of 8.0 to 8.4. Lake Superior water at a hardness of 45.5 ± 1.6 mg/L, alkalinity of 44.1 ± 2 mg/L, conductivity of 95.4 ± 3.7 µmhos/cm, and pH of 7.44 to 8.25 was used for all tests with fathead minnows, gammarids, and midges. Medium T82MV⁶ was used for microcosm tests. The addition of polyelectrolytes did not significantly change these general water quality parameters at concentrations where LC₅₀s were determined. These measurements were not made if the compound was not toxic at 100 mg/L. Dissolved oxygen was maintained near saturation.

Polyelectrolytes were weighed on an analytical balance and gently poured into a vortex of water using a magnetic stirring bar. They were then stirred until dissolved. All concentrations given are amounts added. For tests with clays, a turbidimeter was used to determine the concentrations. The clays were stirred with a magnetic stirring bar; the polyelectrolyte in water was then added to each test container. These mixtures were stirred for 10 minutes and allowed to precipitate for 1 hour before adding the test organisms.

The microcosm test was started as prescribed by Taub⁶ using daphnids, rotifers, *Hyalella*, ostracods, protozoans, and the algal species listed. However, most of the chemical measurements were not made as prescribed in the protocol. Animal and algal counts were not made as frequently as called for in the protocol, and the number of replicates was reduced from six to two, except for the controls.

Nonionic and anionic polymers were not acutely toxic to the four species tested; cationic polymers were very toxic and their toxicity varied widely with their chemical structure.

Microcosms were reinoculated every week with larger organisms (daphnids, *Hyalella*, and ostracods) if they were all dead. One drop (approximately 0.05 mL) of a mixture of the ten algal species, one drop of the rotifers and one drop of the protozoans were also added. The amount of algae added was below detection limits. The reinoculation was to reduce extinctions and variance

Table 1--Test conditions for acute bioassay experiments.

Species	Stage of development	Test containers	Water volume	Numbers of animals/container	Number tested at each concentration	Test duration
<i>Daphnia magna</i> (daphnids)	Neonates < 24 hrs old	100-mL beakers	80 mL	5	20	48 hours
<i>Pimephales promelas</i> (fathead minnows)	Yolk fry < 24 hrs old	100-mL beakers	80 mL	5	20	96 hours
<i>Gammarus pseudolimnaeus</i> (gammarids)	Adults	1000-mL beakers	500 mL	5	10	96 hours
<i>Paratanytarsus</i> (midges)	3rd-4th instar	100-mL beakers	80 mL	5	10	48 hours

Table 2--The acute toxicity of cationic polyelectrolytes to selected aquatic organisms (values in mg/L).

Polyelectrolytes	Daphnids 48-hr LC ₅₀	Fathead minnows 96-hr LC ₅₀	Midges 48-hr LC ₅₀	Gammarids 96-hr LC ₅₀
Polymer A	>100	>100		
Polymer B	0.77 (0.40-1.48) ^a	>100	>100	31.6 (9.7-102.9)
Polymer C	>100	>100		
Polymer D	>100	7.4 (6.5-8.5)	>100	102.9 (63.3-167.3)
Polymer E	1.2 (1.1-1.4)	0.88 (0.80-0.97)	26.9 (14.1-51.1)	22.8 (13.4-38.7)
Polymer F	0.24 (0.19-0.32)	2.87 (2.47-3.34)	50.0 (37.2-67.3)	>100
Polymer G	0.32 (0.25-0.42)	1.00 (0.36-1.17)	<6.25	8.10 (5.15-12.75)
Polymer H	0.71 (0.49-1.04)	2.46 (2.16-2.81)	>100	>100
Polymer I	0.13 (0.11-0.16)	3.74 (3.45-4.16)	>100	>100
Polymer J	6.78 (2.79-17.46)	9.47 (8.63-10.40)		112.25 (74.25-169.70)
Polymer K	0.09 (0.05-0.15)	6.82 (6.38-7.31)		>100
Polymer L	1.84 (1.34-2.51)	5.7 (5.3-6.2)		33.4 (15.0-74.3)
Polymer M	12.59 (7.05-22.48)	2.18 (1.86-2.54)	>100	21.0 (11.7-37.7)
Polymer N	70.71 (55.01-90.90)	2.72 (2.36-3.13)		85.2 (62.4-116.4)
Polymer O	0.50 (0.41-0.61)	1.05 (0.92-1.20)		12.5 (8.3-18.9)

^a Numbers in parentheses represent 95% confidence limits as determined by the Trimmed Spearman-Kärber method.⁸

among replicates and is the ecological equivalent of immigration.⁶

Tests for gill damage were made with 30-day-old fish in battery jars using 1 L of water. The water and polyelectrolyte solutions were renewed daily by siphoning most of the water out and then adding 1 L of freshly prepared solution. Whole fish from these exposures were anesthetized in tricaine methanesulfonate and killed in 10% neutral buffered formalin. The heads were then severed from the body posterior to the gills. Heads were dehydrated in graded ethanols, embedded in plastic and sectioned using glass knives. Sections were cut at 3 μ thickness, floated on warm water, picked up on glass slides, oven-dried at 56°C overnight, and stained with methylene blue-azure II-basic fuchsin.⁷ Because of the small specimen size, sections were checked frequently with a microscope to be certain gill tissue was present. Slides were examined and photographed with a photomicroscope. Statistical analyses of acute tests were made using the Trimmed Spearman-Kärber Method for determining LC₅₀ values and 95% confidence limits.⁸

RESULTS

The acute LC₅₀s of three nonionic polyelectrolytes tested with daphnids and fathead minnows were greater than 100 mg/L. Of 10 anionic compounds tested, one experimental polyelectrolyte was toxic to daphnids but not to fathead minnows. Acute toxicities of the fifteen cationic polyelectrolytes tested to daphnids, fathead minnows, midges and gammarids are given in Table 2. The LC₅₀s for two of these were greater than 100 mg/L for daphnids and fathead minnows. However, the others had LC₅₀s of 0.09 to 70.71 mg/L for daphnids, and 0.88 to 9.47 mg/L for fathead minnows. Midge LC₅₀s were less than 100 mg/L for three of eight cationic polyelectrolytes tested (less than 6.25 to 50 mg/L). The LC₅₀s for gammarids were 8.1 to 33.4 mg/L for 7 of 13 cationic polyelectrolytes tested. In general, polyelectrolytes were more toxic to daphnids or fathead minnows than to midges or gammarids.

Acute tests to determine the detoxification of a cationic polymer by an anionic polymer were made with fathead minnows and daphnids (Table 3). The cationic polymer was added at a

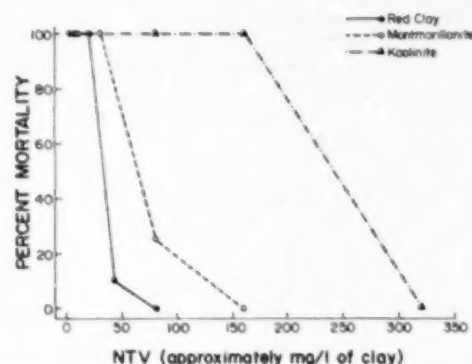


Figure 1—Detoxification of 5 mg/L of Polymer O by three kinds of clay to fathead minnows.

concentration of 10 mg/L, approximately 8 times the LC₅₀ for fathead minnows and approximately 11 times the LC₅₀ for daphnids. The cationic polymer was completely detoxified to fathead minnows by 20 mg/L of the anionic polymer. To find the anionic polymer concentration required to detoxify the cationic polymer to daphnids, four concentrations of the anionic polymer were added along with 10 mg/L of cationic polymer. Approximately the same amount of the anionic polymer (about 11 mg/L) was needed to detoxify the cationic polymer (Table 3).

A red clay (composed primarily of illite and chlorite), montmorillonite clay, and kaolinite clay were tested at several concentrations in separate experiments to determine the amount of clay needed to detoxify a cationic polyelectrolyte added at a concentration of 5 mg/L (approximately 5 times the LC₅₀) to fathead minnows (Figure 1). The amounts of clay needed to detoxify the cationic polyelectrolyte were 80 mg/L, 160 mg/L, and 320 mg/L respectively for red clay, montmorillonite clay and kaolinite clay.

In the microcosm tests, the total number of algae in the medium and high polyelectrolyte concentrations peaked at a later time than in the controls and low polyelectrolyte concentration (Figure 2). In the high concentration the number of algae increased slowly at first because many of the algae were probably flocculated by the polyelectrolyte. However, algal numbers were very high by Day 48. This may be attributed to the low daphnid numbers (Figure 3). Reinoculated daphnids did not live until after Day 40 at which time they started to multiply and crop the algae. Daphnids were affected by the medium and high polyelectrolyte concentrations.

The ostracod population was adversely affected only in the high polyelectrolyte concentration by the end of the test, although less pronounced effects were noted at other concentrations on other sampling days (Table 4). *Hyaella* were adversely affected at all concentrations of the polyelectrolyte tested (Table 5). A decrease in population density was noted with each concentration tested and was particularly evident on Day 63. Rotifers and protozoa were often not found in the microcosms and their numbers were sporadic. These variations could not be consistently associated with polyelectrolyte concentrations.

Algal species composition differed among treatment groups because of the interaction of several factors including possible direct effects of the polyelectrolytes and indirect effects (Table

Table 3—Detoxification of a cationic polymer by an anionic polymer.

Concentrations in mg/L		Mortality	
Cationic polymer	Anionic polymer	Fathead minnows in 96 hrs.	Daphnia in 48 hrs.
0	0	0%	0%
0	20	0%	0%
10	0	100%	100%
10	20	0%	—*
10	3	—	45%
10	7	—	5%
10	11	—	0%
10	17	—	0%
10	26	—	0%
0	26	—	0%

* Not tested.

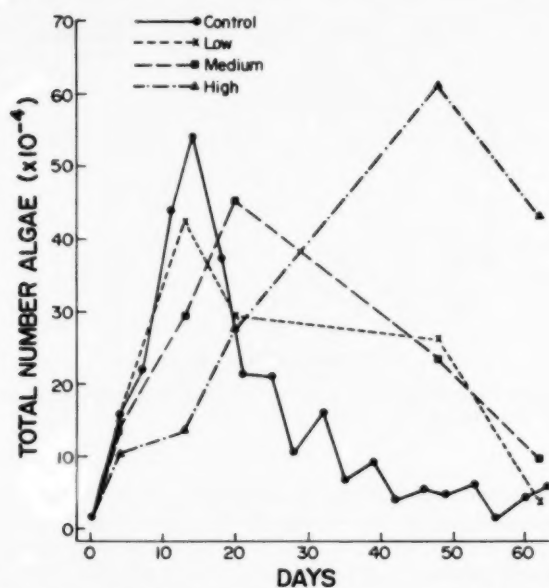


Figure 2—The effect of Polymer E on algae in microcosms. Low, medium and high concentrations equal 1.34, 13.4 and 31 mg/L, respectively.

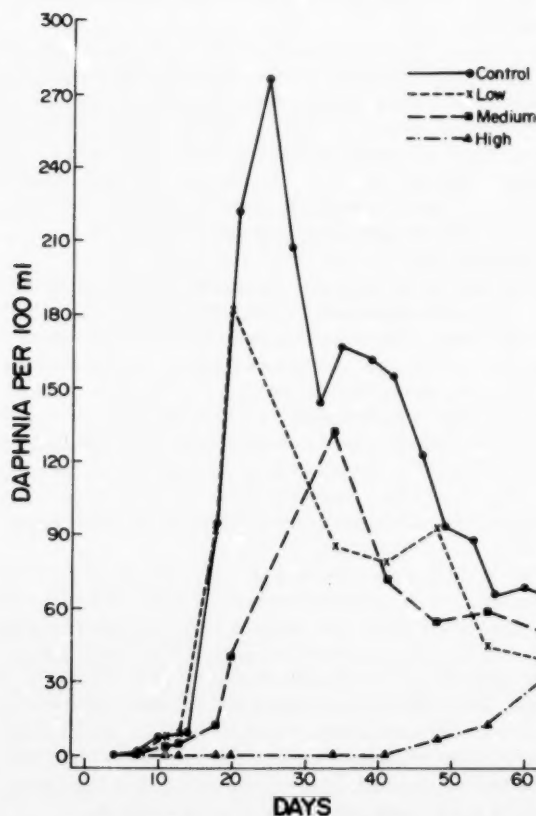


Figure 3—The effect of Polymer E on *Daphnia magna* in microcosms. Low, medium and high equal 1.34, 13.4 and 31 mg/L, respectively. Median ratio of daphnia counts in duplicate jars is 1.4.

Table 4—Ostracod population density in microcosms (mean numbers per 100 mL). Median ratio of ostracod counts in duplicate jars was 1.4.

Day	Control	Concentrations of polymer E in mg/L		
		1.34	13.4	31.0
11	0.06	0.10	0.04	0.00
13	0.07	0.10	0.00	0.00
20	0.01	0.00	0.00	0.00
41	1.72	0.23	0.11	0.00
48	7.50	2.50	3.00	0.00
55	9.33	9.50	5.00	0.58
63	12.83	10.75	14.50	2.00

6) induced by changes in animal numbers and species. The low number of daphnids and *Hyalella* throughout the test in the polyelectrolyte treatments probably influenced the species composition. On Day 48 species diversity seemed to be normal in the controls and the two lower concentrations tested. By Day 62, *Scenedesmus* comprised 98.02% of the algae in the high concentration; however, many other species were present in controls and the two lower concentrations.

Microscopic examination of gills from fathead minnow controls showed a regularity of filament structure with red blood cells in the lamellar capillaries and a thin layer of respiratory epithelium covering the lamellae (Figure 4). After a 24-hour exposure to 0.5 mg/L of Polymer E, the lamellar epithelium was thickened and exhibited a "fuzzy" appearance, either from accumulated mucus or the polyelectrolyte itself. After 96 hours, the lamellar structure was still visible along the gill filament, but cells were accumulating in the interlamellar spaces and the tips of the filaments were completely covered by infiltrating cells (Figure 5).

Gills from fish exposed to 1.0 mg/L of Polymer E had a marked increase in interlamellar cell masses after only 24 hours, with white blood cells, mucous cells, and chloride cells present. This cellular infiltration continued, and after 48 hours cell masses extended to the tips of the lamellae in some areas. Lamellae that

Table 5—*Hyalella* population density in microcosms (mean numbers per 100 mL). Median ratio of *Hyalella* counts in duplicate jars was 1.2.

Day	Control	Concentration of polymer E in mg/L		
		1.34	13.4	31.0
11	0.18	0.18	0.14	0.12
13	0.20	0.17	0.13	0.13
18	0.15	0.08	0.11	0.03
20	0.15	0.12	0.14	0.03
34	0.26	0.05	0.06	0.00
41	0.23	0.20	0.09	0.03
48	0.34	0.12	0.07	0.04
55	0.53	0.16	0.12	0.04
63	1.89	0.77	0.38	0.25

Table 6—Mean algal genera composition by percent in microcosms.

Day	Genera	Control	Concentrations of polymer E in mg/L		
			1.34	13.4	31.0
4	Anabaena	0.80	7.87	0.00	2.17
	Ankistrodesmus	23.24	21.62	25.08	27.64
	Chlamydomonas	33.58	12.55	33.26	28.54
	Chlorella	5.54	13.22	8.90	3.14
	Lyngbya	5.23	18.08	0.00	0.00
	Nitzschia	6.84	4.57	5.31	7.60
	Scenedesmus	4.33	2.92	5.92	7.55
	Selanastrum	14.25	18.47	16.84	13.78
	Stigeoclonium	0.00	0.39	0.00	0.00
	Ulothrix	6.17	0.30	4.68	9.58
20	Anabaena	7.23	0.00	5.79	0.00
	Ankistrodesmus	18.88	6.97	12.63	2.52
	Chlamydomonas	6.24	3.68	32.28	36.31
	Chlorella	3.23	3.44	2.69	2.57
	Lyngbya	10.38	0.00	0.00	0.00
	Nitzschia	29.97	61.63	4.26	8.03
	Scenedesmus	9.84	9.94	30.08	47.83
	Selanastrum	14.24	14.34	9.84	1.79
	Stigeoclonium	0.00	0.00	1.12	0.00
	Ulothrix	0.00	0.00	1.30	0.94
48	Anabaena	1.00	6.59	0.00	0.00
	Ankistrodesmus	7.61	23.86	16.32	0.32
	Chlamydomonas	4.49	7.04	16.30	1.99
	Chlorella	0.00	1.32	2.17	0.48
	Lyngbya	11.48	13.62	16.60	10.21
	Nitzschia	52.70	21.29	16.53	0.86
	Scenedesmus	1.00	8.63	22.06	85.53
	Selanastrum	2.75	16.53	5.88	0.51
	Stigeoclonium	18.97	1.13	4.13	0.08
	Ulothrix	0.00	0.00	0.00	0.00
62	Anabaena	0.00	0.00	8.64	0.00
	Ankistrodesmus	0.40	24.11	4.41	0.04
	Chlamydomonas	2.78	4.57	6.95	0.53
	Chlorella	0.20	0.51	1.36	0.00
	Lyngbya	59.98	26.65	58.13	0.72
	Nitzschia	8.94	17.00	5.76	0.53
	Scenedesmus	0.60	5.08	10.00	98.02
	Selanastrum	0.60	6.09	1.02	0.15
	Stigeoclonium	26.51	15.99	3.73	0.00
	Ulothrix	0.00	0.00	0.00	0.00

were still relatively free of cell infiltration were thickened and bent.

Gills from fish exposed to 2.0 mg/L of Polymer E for 24 hours showed cellular infiltration, especially at the tips of the filaments, which were full of cells and covered with a layer of epithelium. The branching filament structure was still visible, but capillary spaces and red blood cells were not apparent. Mucous cells were evident, especially near the filament tips (Figure 6).

After a 24-hour exposure to 3.0 mg/L of Polymer E, gills from surviving fish had very little lamellar structure. The entire filament was a mass of cells surrounded by a layer of epithelium. Several areas within the filament had no definable structure left, with cell fragments and debris present. Debris was also accu-



Figure 4—Fathead minnow gills. Control fish. Note regularity of gill structure with red blood cells in lamellar capillaries and single layer of thin epithelial cells covering the lamellae. 160X.

mulated on the surfaces and between the gill filaments (Figure 7).

DISCUSSION

The nonanionic and anionic polyelectrolytes tested were not acutely toxic at 100 mg/L to four species of aquatic animals with the exception of one experimental anionic polymer. Biesinger *et al.*¹ reported *Daphnia magna* 96-hr TL_{50} s of an anionic and nonionic polymer to be 17.0 mg/L and 16.5 mg/L, respectively. The KGK⁴ reported 96-hr TL_{50} s of two anionic polyelectrolytes to be 53.2 mg/L and 75.2 mg/L, respectively, to rainbow trout (*Salmo gairdneri*). Very few of these compounds have been tested and thus should not be considered innocuous.

Of the 15 cationic polyelectrolytes tested, only two were not toxic at 100 mg/L. The LC_{50} of Polymer B was 0.77 mg/L for daphnids and greater than 100 mg/L for fathead minnows, whereas the LC_{50} of Polymer D was greater than 100 mg/L for

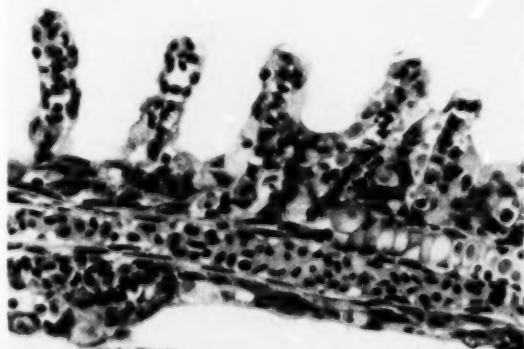


Figure 5—Fathead minnow gill after 96-hour exposure to 0.5 mg/L Polymer E. Lamellar structure still visible along gill filament. Interlamellar spaces filled with cells. An epithelial layer surrounds lamellae. 160X.

daphnids and 7.5 mg/L for fathead minnows. The large differences in sensitivity (see Table 2 for other examples) between these species suggest different modes of toxic action. In addition to suffocation, oligomers present as impurities could be causing toxic effects. It is also apparent from Table 2 that one cannot test one species and predict safe concentrations for other species. The results given in this paper and elsewhere^{1,4,9} show that many of the cationic polyelectrolytes are very toxic; however, there are large differences in the toxicity of various formulations which is probably accounted for by their chemical structure.

Fathead minnow fry were used for all acute tests; older fish were used for histological work. The LC_{50} of Polymer E for fathead minnow fry was 0.88 mg/L; a bioassay with 45-day-old fathead minnows gave a 96-hr LC_{50} of 2.83 mg/L; this suggested that young fry are more sensitive than older fish.

Polyelectrolytes are long chain molecules and those with the longest chains are more effective as flocculants. These molecules adsorb on particles in suspension and then bridge into agglomerates or flocs. When particle concentrations are too low to flocculate all of the polymer, excess polymer molecules can adsorb on gill surfaces. Yamamoto¹⁰ suggested that the gills of medaka were negatively charged and thus attracted a cationic polymer. Large molecules of the polymer may also act as physical irritants because of their size.

McDonald⁹ showed that fish survived in a suspension of cationic polyelectrolyte as long as particles were present to adsorb the polymer. In fact, some fish populations seemed healthier and had increased feeding activity after flocculants were used to decrease turbidity in hatchery ponds. However, fish mortality occurred if flocculants were added when turbidity was low. KGK⁴ reported white, solid matter on carp gills in an aqueous solution of cationic polyacrylamide and concluded that the animals died from suffocation caused by the formation of a film on gills of test fish.

The interlamellar cell masses in fathead minnow gills exposed to Polymer E proliferated until the lamellae were fused and the diffusion distance between oxygen in the water and red blood cells in the gill capillaries was increased. In addition, as the ir-

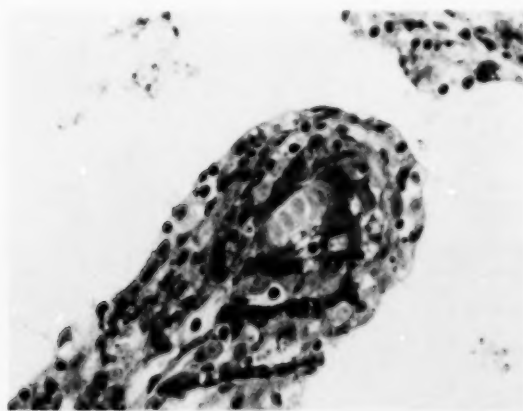


Figure 6—Fathead minnow gills after 24-hour exposure to 2.0 mg/L Polymer E. Lamellae visible along gill filament. Interlamellar spaces completely filled with cells. Entire filament has a surrounding layer of epithelium. 160X.

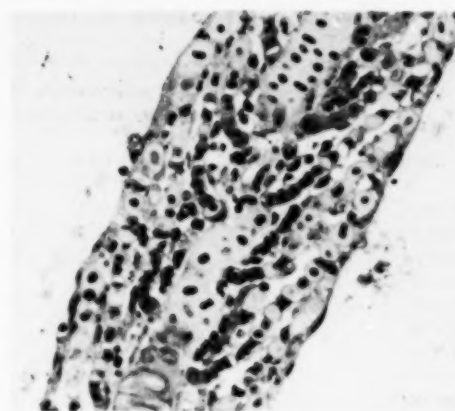


Figure 7—Fathead minnow gill after 24-hour exposure to 3.0 mg/L Polymer E. Some lamellar structure remains. Areas of cell breakdown with no definable structure. Debris adhering to layers of epithelium that surrounds filament. 160X.

ritation or agglomeration continued, a layer of epithelium covered the entire filament, and added another barrier to efficient respiration. As the lack of oxygen became more acute, cell death occurred, and with longer exposures or higher concentrations of the polymer, breakdown of the entire lamellar structure was evident. These fish did indeed suffocate as a result of cellular response to the presence of the polymer.

The tests showing that a cationic polyelectrolyte will detoxify an anionic one confirm both a Japanese¹¹ and German patent.¹² Cationic polyelectrolytes could very easily be discharged into natural waters at concentrations that could affect aquatic animals. To prevent this, an anionic polyelectrolyte can be added before discharging wastewater. Detoxification of the cationic polyelectrolyte by three kinds of clay was expected. Differences in the negative charges on the three kinds of clay particles account for the various amounts needed to satisfy the positive charges on the polymer.

In the microcosms, algal growth was delayed at the higher polyelectrolyte concentrations. Algal cells were possibly bound by the polymer and thus could not reproduce until all charges were satisfied. It was not evident that the polymer caused a direct toxic effect on the algae. Differences in species composition over time in the treated versus the untreated microcosms could be partially related to the polymer, but differences in grazing cannot be ruled out.

The low concentration tested in the microcosm was the 48-hr LC_{50} for daphnids and did not affect them initially, presumably because the charges on the polymer were neutralized by the algae. Only the high concentration prevented daphnids from living until after Day 40.

ACKNOWLEDGMENTS

Authors. Kenneth E. Biesinger and Gertrude N. Stokes are biologists at the Environmental Research Laboratory-Duluth, U. S. Environmental Protection Agency. Correspondence should be sent to Kenneth E. Biesinger, 6201 Congdon Blvd., Duluth, MN 55804.

REFERENCES

1. Biesinger, K. E., *et al.*, "Comparative toxicity of polyelectrolytes to selected aquatic animals." *J. Water Pollut. Control Fed.* **48**, 183 (1976).
2. Dow Chemical Co., "Chemical Treatment of Combined Sewer Overflows." U. S. Environmental Protection Agency, Water Poll. Control Res. Ser. 11023FDB09/70, Washington, D. C. (1970).
3. Brocksen, R. W., "An Evaluation of Potential Sources of Toxicity to Fish in Martis Creek." U. S. Army Corps of Engineers, Sacramento District, Calif. (1971).
4. Kobunshi Gyoshuzai Konwakai (High Molecular Flocculant Association). "Safety of Polyacrylamide Flocculants and Safety of Cationic Polyacrylamide Flocculants." (Mimeo).
5. "Standard Methods for the Examination of Water and Wastewater," 15th Ed., Am. Public Health Assoc., Washington, D. C. (1980).
6. Taub, F. B., and Read, P. L. "Standardized Aquatic Microcosm Protocol." (Mimeo) 1983.
7. Humphrey, C. D., and Pittman, F. E. "A Simple Methylene Blue-azure II-Basic Fuchsin Stain for Epoxy-embedded Tissue Sections." *Stain Technology* **49**, 9 (1974).
8. Hamilton, M. A. *et al.*, "Trimmed Spearman-Kärber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays." *Environ. Sci. Technol.* **12**, 417 (1978).
9. McDonald, R. A., "Use of a Polyelectrolyte to Reduce Soil Turbidity in Two Fish Ponds and Effects on Plankton, Benthos and Fishery." Unpubl. (1971).
10. Yamamoto, S., "Safety of Cationic Polymeric Coagulants." *Seikatsu to Kankyo* **24**, 8, 32 (1979).
11. Furuno, A. *et al.*, "Method of Rendering Cationic Polymers Harmless to Fish." Japan Patent Agency Patent Gazette. Kokai No. 1976-454-51.
12. German Patent Application 29-42-111. "Process for the Detoxification of Water Treated with Polymer Cationic Flocculation Agents." (Mimeo) 1980.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.